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Non-Radioactive TRF Assay Modifications to Improve Telomeric DNA Detection Efficiency in Plants

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Abstract

© 2016, Springer Science+Business Media New York.The length of telomeric DNA is often considered a cellular biomarker of aging and general health status. Several telomere length measuring assays have been developed, of which the most common is the telomere restriction fragment (TRF) analysis, which typically involves the use of radioactively labeled oligonucleotide probes. While highly effective, this method potentially poses substantial health concerns and generates radioactive waste. Digoxigenin (DIG) alternatives to radioactive probes have been developed and used successfully in a number of assays. Here, we optimize the DIG protocol to measure telomere length in the model plant Arabidopsis thaliana and present evidence that this approach can be used successfully to efficiently and accurately measure telomere length in plants. Specifically, hybridization temperature of 42 °C instead of the typical 55 °C appears to generate stronger signals. In addition, DIG incorporation at 5′-end instead of 3′-end of the labeled oligonucleotide greatly enhances signal. We conclude that non-radioactive TRF assays can be as efficient as radioactive methods in detecting and measuring telomere length in plants, making this assay suitable for medical and research laboratories unable to utilize radioactivity due to hazardous waste disposal and safety concerns.

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Keywords

Aging, DNA, Non-radioactive telomere restriction fragment analysis, Plant, Southern blot